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Two-dimensional analysis of gliadin proteins associated with quality in durum wheat: chromosomal location of genes for their synthesis

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Summary. Two-dimensional electrophoresis was used to fractionate the gliadin proteins from the endosperm of durum wheat. The increased resolution of the system, as compared with single-dimensional analysis, accentuated the heterogeneity of the proteins. This resolution, coupled with the use of aneuploid lines of the cultivar 'Langdon', permitted identification of the chromosomes controlling synthesis of the major protein components. Homoeologous Group 1 chromosomes controlled omega- and gamma-gliadin synthesis and the Group 6 chromosomes 6A and 6B controlled alpha- and beta-gliadins. Chromosome 1B was primarily responsible for the two groups of protein-polypeptides associated with strong or weak gluten characteristics of durum wheat. However, some of these proteins were controlled by chromosome 1A. In the beta-gliadin region several hybrid bands, whose chromosomal control was not identified by electrophoresis alone, were specified primarily by genes on chromosome 6B, although chromosome 6A was also involved. Control of some other hybrid bands could not be determined. Chromosomes in Groups 2, 3, 4, 5 and 7 were not implicated in the synthesis of the gliadin proteins of durum wheat.

Key words: *Triticum turgidum* L. group *durum* – Gliadin – Chromosome – Two-dimensional electrophoresis

Introduction

Evidence is accumulating of a close relationship between the presence of a specific gliadin protein, designated band 45 on its electrophoretic mobility, and strong gluten in durum wheat, (*Triticum turgidum* L. group *durum*). Another protein (band 42) appears to be associated with weak viscoelastic properties (Damidaux et al. 1978, 1980a; Kosmolak et al. 1980). These results were extended by the demonstration that, in over 100 durum genotypes, a group of gliadins (group 45), not just one protein, was associated with strong dough characteristics and another group (group 42) with dough weakness (du Cros et al. 1982).

Damidaux et al. (1980b) also found close associations between a few gliadins and band 42 and between one other gliadin and band 45, and attempted to establish the chromosomal location of genes controlling their synthesis. They reported that the proteins associated with gliadin 42, as well as gliadin 42 itself, were coded for by genes on chromosome 1B. Joppa et al. (1983) performed polyacrylamide gel electrophoresis (PAGE) on gliadin proteins from the endosperm of a set of 'Langdon' durum disomic-substitutions and determined the chromosomal location of genes coding for many of these polypeptides. However, the chromosomal location of genes coding for some gliadin-polypeptides could not be determined because the proteins could not be separated by the methods used and some bands appeared to be controlled by more than one chromosome. One of these bands is associated with gliadin 42 (du Cros et al. 1982). Aneuploids of durum wheat possessing the group-45 proteins are not presently available. However, Damidaux et al. (1980b) reported that band 42 and band 45 appeared to be controlled by alleles at the same locus, or at closely linked loci. Joppa et al. (1983) substituted the 1B chromosome from 'Edmore', a strong gluten cultivar (with band 45), into 'Langdon', a cultivar having poor dough strength (and band 42), and established that both band 45 and band 42 are controlled by genes on chromosome 1B.

Thus, single-dimension PAGE has proved valuable in the identification of chromosomes controlling the synthesis of certain gliadin proteins. This system, however, is limited to identification of cases where a chromosome has sole control over the synthesis of a particular band. Difficulties arise when two, or more, chromosomes are involved in the production of a single-dimension protein band. Two-dimensional electrophoresis provides greatly improved resolution of these protein components. The system generally involves isoelectric focusing in the first dimension, where the proteins are separated according to their overall charge. This is followed by either PAGE or sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension, where the proteins are separated on the basis of charge and size or size alone. Thus, protein bands, which appear to be single polypeptides in one dimension but which are actually composed of two or more proteins, will be resolved into their component polypeptides in the two-dimensional system. This type of analysis was used in aneuploid studies of hexaploid wheat (Wrigley 1970; Wrigley and Shepherd 1973; Brown and Flavell 1981), and has clarified interpretation of the results in a way not possible by electrophoresis in one dimension.

In the study reported here, we have used two-dimensional electrophoresis to examine the chromosomal location of genes coding for proteins, particularly those associated with poor quality, in durum wheat. This resulted in better resolution of the gliadin proteins, partial characterization according to isoelectric properties, and consequently improved understanding of the relevant gene locations.

Materials and methods

Grain samples

The Australian cultivar 'Durati', representing a gliadin band 45 durum wheat (45-type), and the cultivar 'Leeds' (42-type), from the USA were examined to determine the complexity of the proteins associated with quality. Other cultivars studied were 'Langdon' durum (42-type with weak gluten) and the hexaploid wheat 'Chinese Spring'.

Chromosomal locations of genes coding for the quality-related proteins were studied using a set of 'Langdon' disomic-substitution lines in which individual D-genome chromosomes from 'Chinese Spring' replace their A- or B-genome homoeologues (Joppa et al. 1978, 1983). The disomic-nullisomic combinations 4D(4B) and 7D(7A) were not available for examination. A line, in which chromosome 1B from 'Edmore' (a 45-type durum with strong gluten) was substituted into 'Langdon' by crossing and backcrossing to the 'Langdon' 1D(1B) line, was also examined.

Cytology

Several of the substitution lines must be maintained by carrying one of the substituted 'Langdon' chromosomes in the monosomic or telosomic condition. Hence the appropriate plants with 2n = 28 (and presumed to represent 14 chromosome pairs) were identified by somatic chromosome counts on root tips. Kernels from lines being tested were cut in halves, the distal portions reserved for electrophoresis, and the embryo halves germinated. Somatic chromosome numbers were determined using the Feulgen-acetocarmine squash technique. After germination, root-tips were collected and placed in ice-water for 24 h. The root-tips were fixed in alcohol : acetic acid (3 : 1) solution for 24 h and then hydrolysed in 1N HCl in a water bath for 11.5 min at 56 °C. After staining in leuco-basic Fuchsin for at least 20 min the root tips were squashed in acetocarmine and examined under the light microscope. Only half-portions from those kernels containing embryos with 2n = 28 were examined electrophoretically.

Extraction and electrophoresis

The distal halves of selected kernels were crushed and the gliadin proteins extracted overnight at 25 °C in 6% urea (6 µl/mg grain). For examination of cultivars, ground wholemeal samples were extracted.

Single-dimensional PAGE of cultivars was carried out, with slight modifications to the gels, as described below for electrophoresis in the second-dimension. The gel slabs measured approximately 140 × 50 × 1 mm and were cast with a polyacrylamide gradient ranging from 3% at the top to 15% at the bottom; sample slots were formed using a 3% gel.

a) Isoelectric focusing. Glass tubes (70 mm long; 3 mm i.d.) were filled to 60 mm with 6% polyacrylamide solution containing 6% urea and 0.5% carrier ampholytes (Pharmacia AB, Uppsala, Sweden) in the range pH 5 to pH 9. The pH gradient was prefocused for 30 min at 400 V with 0.2% sulphuric acid at the upper electrode (anode) and 0.4% ethanolamine at the lower electrode (cathode). The protein extracts were clarified by centrifugation at 7,500 g for 15 min, 50–60 µl of each extract applied and electrofocusing was continued for 2.5 h at 400 V. The gels were rimmed from their glass tubes and immediately used for protein separation in the second dimension.

b) Gradient gel preparation and electrophoresis. The polyacrylamide gel slabs (165 × 135 × 3 mm) were cast in taped glass cassettes using a multichannel peristaltic pump and an equal quantity (90 ml) of two solutions with acrylamide concentrations of 3% and 11%, respectively. Each solution contained sodium lactate buffer (0.004 M Na, pH 3.1), 0.04% N,N,N',N'-tetramethylethylenediamine (TEMED), and 0.004% ammonium persulphate. Two gels were cast simultaneously in a gel slab-casting apparatus using pump tubes of (a) 2 ml/min and (b) 4 ml/min capacity. To ensure polymerization and a flat gel top, a small amount of diluted buffer (1:10) was initially pumped into the apparatus. The higher concentration acrylamide solution was then pumped via tube (a) to the low concentration solution, which was stirred continuously. At the same time this solution was pumped through tube (b) to the bottom of the casting apparatus to form the gradient. After all of the acrylamide solution had been pumped into the apparatus, 20% glycerol solution was pumped in to bring the acrylamide level to the bottom of the gel cassettes. At this stage the overlayering buffer was near the top of the cassettes. Total time for pumping was about 1 h. Polymerization of the gels occurred soon after casting was complete. Gels were always prepared one day before use to ensure complete destruction of unreacted free radicals from the polymerisation reaction.

Electrophoresis in the second dimension was performed in sodium lactate solution (pH 3.1, 0.004 MNa). Two slab gels in

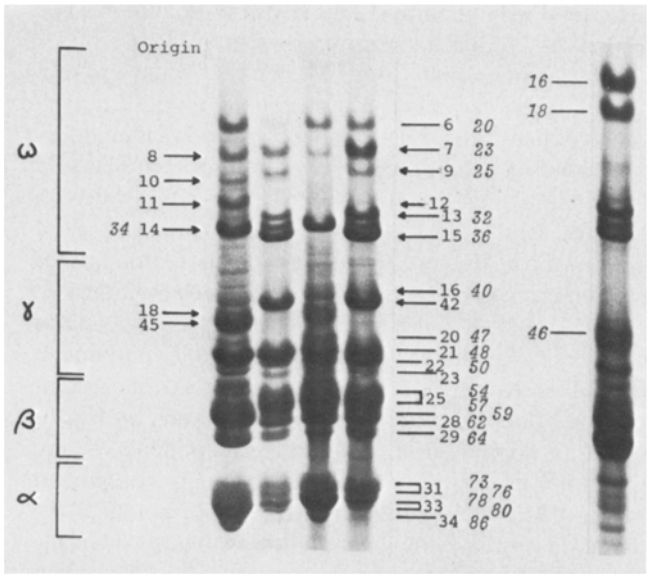

Fig. 1. Gradient gel electrophoresis (pH 3.1) of gliadin proteins of durum wheat cultivars 'Durati' (*left*), 'Leeds', 'Edmore' and 'Langdon'. The hexaploid wheat 'Chinese Spring' is included on the extreme right for comparison. The bands comprising the quality-associated groups 45 and 42 (*left* and *right*, respectively) are *arrowed and numbered* according to du Cros et al. (1982). Where direct comparisons were possible, the designations of Joppa et al. (1983) are listed in italics. Regions for the gliadin proteins are shown on the *left*

their cassettes were loaded into the apparatus and two tube gels from the first dimension separation were placed horizontally end to end on top of each gel slab. A sample guide with two or four positions was inserted between each pair of tube gels and 20 µl of each of the corresponding original protein extracts was loaded into the guide. Reference extracts of 'Langdon' and 'Chinese Spring' were loaded when the disomic-substitution lines were examined. Cathodic electrophoresis was performed at 200 V for 5.75 h. After removal from their glass holders, the gels were stained overnight at 25 °C in 200 ml of 12% trichloroacetic acid solution containing 0.02% Coomassie Blue G250 dye. Destaining was unnecessary but the gels were washed gently with water to remove precipitated dye on the surfaces before being photographed with Ilford 35 mm Pan F film. A red filter was used to enhance the contrast.

Gliadin nomenclature

The nomenclature used throughout this paper for the gliadin bands comprising the quality groups is that of du Cros et al. (1982). A key to the numbering system of Joppa et al. (1983) is shown in Fig. 1 for cases where corresponding bands could be identified.

Results and discussion

Resolution of durum gliadins

By comparison with gel electrophoresis alone, combined isoelectric focusing/electrophoresis improved the resolution of all gliadins (Figs. 1 and 2). Most zones

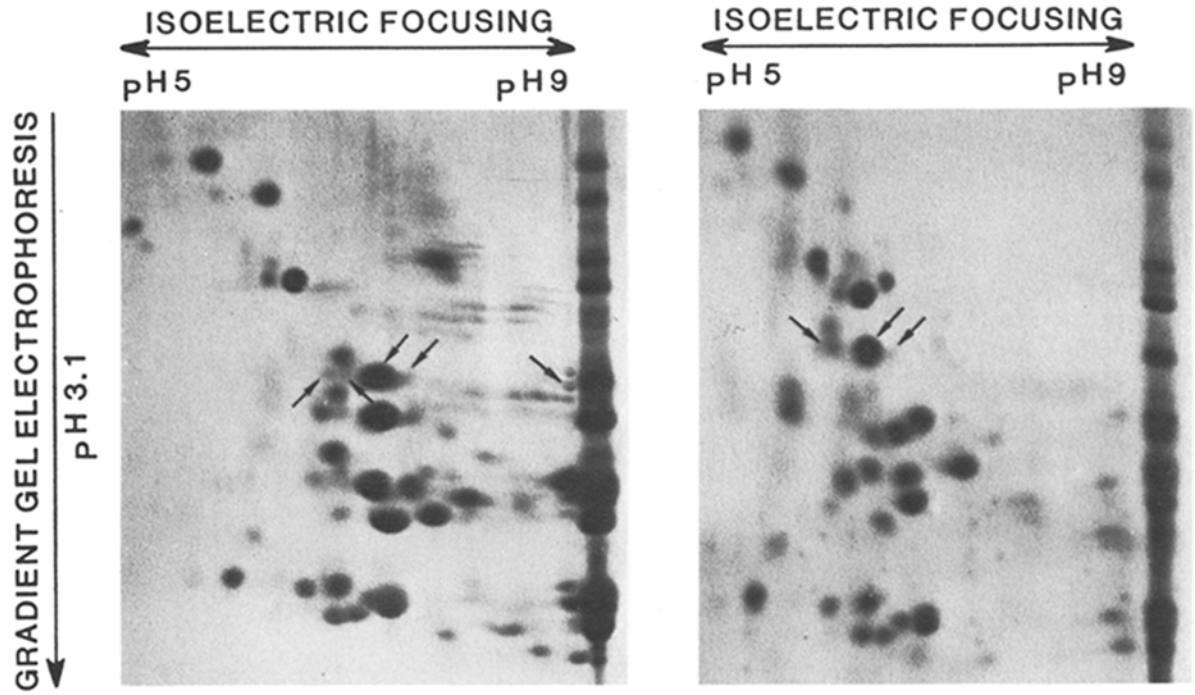

Fig. 2. Two-dimensional analysis, combining isoelectric focusing and gradient gel electrophoresis, of two cultivars, 'Durati' (*left*) and 'Langdon' (*right*). Patterns obtained by electrophoresis alone are shown on the *right* of each protein map. Mobilities in the single dimension analysis are slightly greater than those obtained two-dimensionally because of differences in the manner of sample application (in solution vs in a gel rod). Components of bands 42 and 45 are *arrowed*

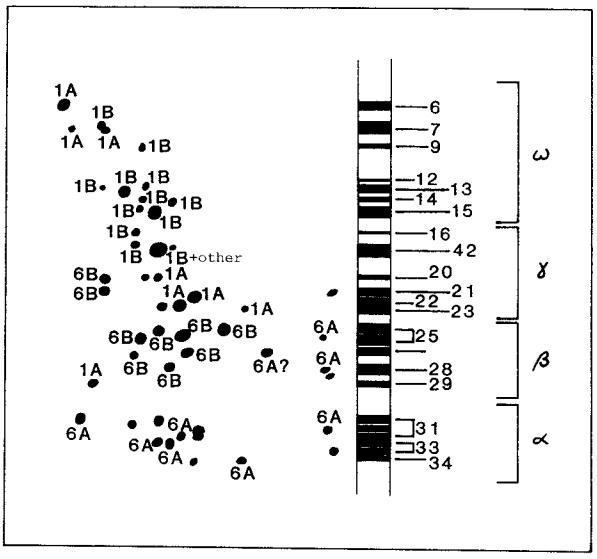

Fig. 3. Diagram of the two-dimensional protein map for 'Langdon' showing the chromosomes controlling synthesis of gliadin components. Band positions (du Cros et al. 1982) are *numbered on the right*

resolved by gel electrophoresis were shown to contain several proteins that differed in isoelectric point. In general, gliadins with low electrophoretic mobilities were more acidic than the alpha- and beta-gliadins which had very basic minor components. Electrophoretic bands of similar mobility sometimes differed from one genotype to another in the numbers of minor components, and in their isoelectric points when compared by two-dimensional analysis.

Particular attention was directed to the composition of gliadins 42 and 45 and to the gliadins previously shown to occur together with one or the other (du Cros et al. 1982). These are arrowed in Fig. 1. Band 14 is associated with gliadin 45, but is also present in 42-type durums at a reduced intensity.

Two-dimensional analysis shows both protein groups to be far more complex than is indicated by single-dimensional electrophoresis (Fig. 2). Band 45 is composed of a major, plus several minor, proteins and other main members of the group, bands 8 and 10, have at least two components each. Band 14, as it appears in a 45-type, consists of a major plus one or two minor components. The main omega-gliadins of group 42, bands 7, 13 and 15, all comprise one major component with at least one minor component. Band 42 consists of one major plus two minor components. Band 14, as it occurs in association with gliadin 42, is composed of three small components, one of which lies in a position similar to the major component of band 14 of 45-type durums. Isoelectric points of the group 42 proteins are generally lower than those of the group 45 proteins.

Chromosomal control

Chromosomes 1A, 1B, 6A and 6B were found to control the synthesis of most gliadin proteins (Fig. 3); chromosomes 1A and 1B were mainly associated with the omega- and gamma-gliadins, whereas 6A and 6B mainly determined the alpha- and beta-gliadins. Clear evidence for the involvement of other chromosomes was not found. These results were obtained by associating the absence of particular components with the absence of a chromosome pair in a particular substitution line (Figs. 4 and 5). The results confirmed the findings of Joppa et al. (1983) and were in agreement with conclusions for hexaploid wheat (Wrigley and Shepherd 1973; Mecham et al. 1978; Wrigley 1982).

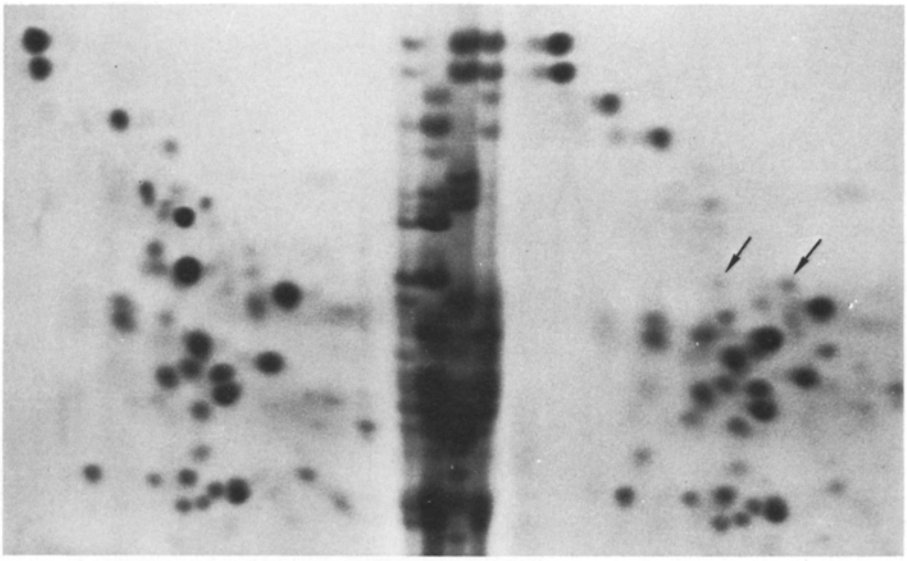

Fig. 4. Two-dimensional maps of the 'Langdon' durum D-genome disomic-substitution lines 1D(1A) (*left*) and 1D(1B) (*right*), showing protein components controlled by genes on the Group 1 chromosomes. The single-dimension patterns are, *from left to right*, line 1D(1A), 'Langdon', 'Chinese Spring' and line 1D(1B)

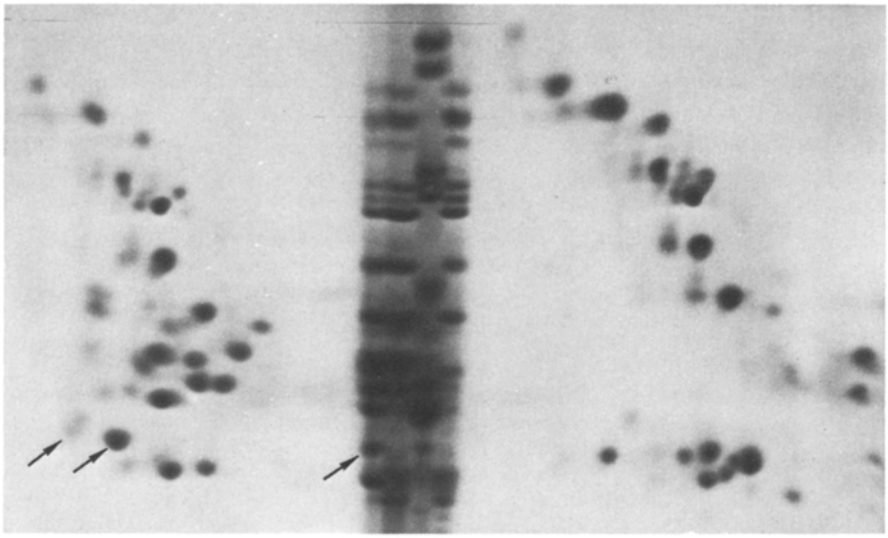

Fig. 5. Two-dimensional maps of the 'Langdon' durum D-genome disomic-substitution lines 6D(6A) (*left*) and 6D(6B) (*right*), showing protein components controlled by genes on the Group 6 chromosomes. The single-dimension patterns are, *from left to right,* line 6D(6A), 'Langdon', 'Chinese Spring' and line 6D(6B)

a) Proteins controlled by homoeologous Group 1 chromosomes. Homoeologous Group 1 chromosomes appear to control the majority of the proteins related to poor viscoelastic properties. Gliadin bands 9, 13, 15 and 16 of the 42 group, and band 14 associated with the 45-group, are controlled solely by chromosome 1B. These bands correspond to bands 25, 32, 36, 40 and 34, respectively, in the system of Joppa et al. (1983) (Fig. 1). Because of their close relationship, shown statistically (du Cros et al. 1982), and their control by one particular chromosome, they may be inherited in a gliadin block, such as those described by Sozinov et al. (1978, 1979) for hexaploid wheat.

Band 42, however, appears to be only partly coded by genes on chromosome 1B. Figure 4 shows the presence of minor components (arrowed) in the protein map of the 1D(1B) substitution line. It could not be determined which other chromosome(s) is involved, because it appears that some spots on the two-dimensional maps are composed of more than one protein. Consequently, the removal of one component is masked by the remaining component(s) and small changes in intensity are difficult to quantify, even with the use of densitometry. These components could be identical proteins or proteins in which point mutations in the amino acid sequence have occurred where the change does not affect the overall charge of the protein. Alternatively, it is possible that genes controlling protein synthesis are duplicated on homoeologous chromosomes. Other faint components close to the position of band 13 (Fig. 4) are coded by genes on the substituted chromosome 1D of 'Chinese Spring', as are the two intensely stained proteins closest to the origin.

The single-dimension band 7, analogous to band 23 of Joppa et al. (1983), is shown by the improved resolution of two-dimensional analysis to be composed of three components close to one another (Figs. 2 and 3). The slower moving component is controlled by chromosome 1B whereas the other two proteins are controlled by the homoeologous chromosome 1A. It is possible that the unidentified components of band 42 are also coded by genes on chromosome 1A.

The cultivar 'Edmore', produced by the North Dakota breeding programme, is a good quality durum wheat with strong viscoelastic properties. Electrophoretic analysis shows it to possess some of the group 45 proteins, namely bands 8, 14 and 45 (Fig. 1). Examination of a line in which chromosome 1B of 'Edmore' had been substituted for its homologue in 'Langdon', indicated that these bands were controlled by the substituted chromosome. It is conceivable that because groups 42 and 45 are allelic, these proteins may also be inherited in a gliadin block similar to that of the group 42 proteins. Because 'Edmore' does not possess the other bands of group 45 (bands 10 and 11), the particular chromosome responsible for their synthesis remains unknown. However, it might be inferred that the Group 1 chromosomes are involved, and chromosome 1B is a likely candidate.

b) Proteins controlled by homoeologous Group 6 chromosomes. Major control of alpha- and beta-gliadins is affected by the group 6 chromosomes. Chromosome 6A mainly controls the alpha-gliadins (Fig. 5). There are, however, some components coded by genes on chromosome 6B. A major protein (arrowed in Fig. 5) between the alpha- and beta-gliadins appears to have come from the 6D chromosome of 'Chinese Spring' as it is absent from the protein pattern of 'Langdon' and is present in 'Chinese Spring'. However, this component is

also absent in the 6D(6B) aneuploid line. Joppa et al. (1983) found a band in a similar position (band 69) in their patterns of both the 6D(6A) and 6D(6B) lines but not in the 'Langdon' 6D disomic-addition line. Possibly, expression of some gliadin protein genes is regulated by other genes, as suggested by Brown et al. (1981). For example, there may be suppression under certain conditions of the gene(s) controlling band 69 by genes on homoeologous chromosomes. A possible cytological basis for these discrepancies is also being investigated.

The hybrid bands 54, 57, 59 and 62 of Joppa et al. (1983) lie in the beta-gliadin region of the protein pattern. These bands appear to be mainly coded for by genes on chromosome 6B (Fig. 5), although minor components with high isoelectric points are controlled by chromosome 6A. Chromosome 6B is also involved in the control of some of the faster-moving gamma-gliadins (Fig. 3); Group 1 chromosomes also code for some of these proteins.

Thus, in durum wheat, chromosome 6A provides the major control of the alpha-gliadins whilst the homoeologous chromosome 6B is involved mainly in the synthesis of the beta-gliadins and partly the gamma-gliadins. This is in accord with results from the study of chromosomal control in hexaploid wheat (Wrigley and Shepherd 1973).

c) Other chromosome groups. Shepherd (1968), Waines (1973) and Brown and Flavell (1981) have implicated Group 2 chromosomes in the control of gliadin proteins in hexaploid wheat. Analysis of aneuploid lines of 'Chinese Spring' showed that, in particular, chromosomes 2A and 2D acted in conjunction with homoeologous Group 6 chromosomes. The durum aneuploids, 'Langdon' 2D(2A) and 2D(2B), were examined for two reasons: firstly, to investigate their possible involvement in the control of gliadin proteins in association with the Group 6 chromosomes; and secondly, to determine whether they provide control or partial control of the hybrid bands 43, 80, 81 and 86. From examination of the two-dimensional protein maps of the substituted Group 2 chromosome lines, it appeared that neither 2A nor 2B was involved in control of gliadin components associated with the Group 6 chromosomes. Furthermore, they did not appear to be involved in control of the hybrid bands.

None of the remaining chromosomes (Groups 3, 4, 5 and 7) was implicated in the control of gliadin proteins in durum wheat. Thus, although two-dimensional analysis gave considerably more information about the chromosomal control of the gliadin proteins than was obtained by single-dimensional electrophoresis, the chromosomes involved in the synthesis of some gliadins remain unidentified.

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